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Bronchoconstrictive properties of inhaled 8-epi-$PGF_{2\alpha}$ in healthy and heaves-susceptible horses

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Abstract – The 8-epi-$PGF_{2\alpha}$ is a marker of oxidative stress which is increased in lungs of asthmatic humans and heaves-susceptible horses. 8-Epi-$PGF_{2\alpha}$ has also been demonstrated to be an in vitro and in vivo bronchoconstrictor in humans and rodents. We hypothesised that inhaled 8-epi-$PGF_{2\alpha}$ was a bronchoconstrictor in healthy and heaves-susceptible horses in clinical remission. The effect on ventilatory mechanics of nebulised 8-epi-$PGF_{2\alpha}$ was compared to that of $PGF_{2\alpha}$ and U46619, a thromboxane A_2 agonist. Pulmonary resistance (R_L) and dynamic compliance (C_{dyn}) were assessed in six healthy horses and in six heaves-susceptible horses in clinical remission before (baseline) and immediately after a single inhalation challenge of 1 mg 8-epi-$PGF_{2\alpha}$, $PGF_{2\alpha}$, or U46619 and placebo. R_L and C_{dyn} were unchanged after inhalation of 8-epi-$PGF_{2\alpha}$ in healthy horses. In heaves-susceptible horses, 8-epi-$PGF_{2\alpha}$ induced a significant increase of R_L and a significant decrease of C_{dyn} when compared to baseline values. Differences between R_L and C_{dyn} values after 8-epi-$PGF_{2\alpha}$ inhalation and those of placebo inhalation were not significant. Differences with healthy horses were not significant. $PGF_{2\alpha}$ and U46619 induced a significant bronchoconstriction in healthy (R_L and C_{dyn}, versus baseline) and heaves-susceptible horses (R_L and C_{dyn}, versus baseline and placebo). The R_L increase in heaves-susceptible horses after $PGF_{2\alpha}$ inhalation was significantly higher than that in healthy horses. Our results suggest that 8-epi-$PGF_{2\alpha}$ is not a bronchoconstrictor in healthy horses, and a bronchoconstrictor far less efficient than $PGF_{2\alpha}$ and U46619 at the same dose in heaves-susceptible horses.

pulmonary function tests / heaves in horses / arachidonic metabolites

Résumé – Propriétés bronchoconstrictives de la 8-épi-$PGF_{2\alpha}$ inhalée par des chevaux sains et des chevaux atteints de pousse. La 8-épi-$PGF_{2\alpha}$ est un marqueur du stress oxydatif dont la synthèse pulmonaire est accrue chez des humains asthmatiques et chez des chevaux atteints de pousse. Il a été démontré in vitro et in vivo que la 8-épi-$PGF_{2\alpha}$ est un bronchoconstricteur chez l'homme et chez certains animaux de laboratoire. Dans l'étude présentée ci-dessous, le pouvoir bronchoconstricteur de la 8-épi-$PGF_{2\alpha}$ a été évalué chez des chevaux sains (n = 6) et chez des chevaux atteints de pousse en

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rémission clinique (n = 6). L'effet d'une administration par nébulisation de la 8-épi-$PGF_{2\alpha}$ sur la mécanique ventilatoire a été comparé à celui d'une administration de $PGF_{2\alpha}$ et de U46619, un agoniste de la thromboxane A_2. La résistance pulmonaire (R_L) et la compliance dynamique (C_{dyn}) ont été mesurées immédiatement avant (T_0) et après la nébulisation de 1 mg de 8-épi-$PGF_{2\alpha}$, de $PGF_{2\alpha}$, de U46619 ou de placebo. L'inhalation de 8-épi-$PGF_{2\alpha}$ n'a pas induit de modifications de R_L et de C_{dyn} chez les animaux sains. En revanche, la 8-épi-$PGF_{2\alpha}$ modifiait la R_L et la C_{dyn} de manière significative chez les animaux poussifs, mais la comparaison avec le placebo et les chevaux sains n'était cependant pas significative. La $PGF_{2\alpha}$ et la U46619 ont induit une bronchoconstriction significative chez les chevaux sains (R_L et C_{dyn}, comparé au T_0) et chez les chevaux poussifs (R_L et C_{dyn}, comparé au T_0 et au placebo). L'augmentation de R_L mesurée chez les chevaux poussifs était significativement plus élevée que celle mesurée chez les chevaux sains. Ces résultats suggèrent que la 8-épi-$PGF_{2\alpha}$ n'est pas un bronchoconstricteur chez le cheval sain et qu'elle est un bronchoconstricteur beaucoup moins efficace que $PGF_{2\alpha}$ et U46619 à la même dose chez le cheval atteint de pousse.

tests de fonction pulmonaire / pousse équine / métabolites de l'acide arachidonique

1. INTRODUCTION

Heaves is a naturally occurring airway hypersensitivity of adult horses to mould spores sharing characteristic features with human asthma, namely, chronic airway inflammation, airway obstruction and airway hyperresponsiveness (AHR) [4, 24, 28]. Airway obstruction and AHR are linked to airway inflammation, which encompasses numerous inflammatory pathways, generating mediators such as cytokines, leukotrienes, prostaglandins and growth factors [4].

Several years ago, the role of prostaglandins generated by the cyclo-oxygenase pathway was investigated by clinical and experimental approaches in heaves-susceptible horses [9, 12, 31]. Bronchoalveolar lavage (BAL) of heaves-susceptible horses in acute crisis has been shown to contain higher concentrations of PGE_2, $PGF_{2\alpha}$ and TxB_2, a thromboxane A_2 metabolite, than BAL of heaves-susceptible horses in remission or BAL of healthy horses [12, 31]. The bronchoconstrictive properties of these inflammatory mediators have been assessed by in vitro experiments performed on equine trachealis muscle and lung parenchyma. The results of this study demonstrated that $PGF_{2\alpha}$ and the synthetic thromboxane A_2 agonist, U44069, were potent in vitro bronchoconstrictors and it was speculated whether the cyclo-oxygenase pathway played an important role in the aetiology of heaves [9]. Gray et al. refuted this hypothesis by a clinical study in which they demonstrated that a non-steriodal anti-inflammatory treatment during an acute crisis of heaves did not decrease airway obstruction [12].

Recently, a cyclo-oxygenase independent pathway, generating biologically active molecules by oxidative stress, has been shown to be involved in airway inflammation [14]. The most important biological product of this pathway is isoprostane, especially 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) [19, 23]. This prostaglandin-like product of lipid peroxidation is a stereoisomere of the prostaglandin $PGF_{2\alpha}$ and is significantly increased by pulmonary oxidative stress in lungs of patients suffering from asthma [21], chronic obstructive pulmonary disease (COPD) [22], interstitial lung disease [20], etc. The bronchoconstrictive properties of this novel prostanoid have been assessed respectively by in vitro and in vivo studies in humans and rodents, and it was concluded that 8-epi-$PGF_{2\alpha}$ is a bronchoconstrictor of considerable importance, which mainly acts as thromboxane agonist through the TP receptor [3, 11, 17, 25].

Similarly to asthmatic humans, the synthesis of 8-epi-$PGF_{2\alpha}$ is significantly increased in lungs of heaves-susceptible horses in acute crisis [18]. In comparison

with healthy horses or heaves-susceptible horses in remission, the increase of 8-epi-$PGF_{2\alpha}$ is of a similar magnitude to that reported for $PGF_{2\alpha}$ and TxB_2 (approximately twofold).

Given that 8-epi-$PGF_{2\alpha}$ is increased in BAL of heaves-susceptible horses in crisis and that it has been shown to be a bronchoconstrictor in humans and rodents [3, 17, 25], we aimed at evaluating its bronchoconstrictive properties in healthy and heaves-susceptible horses by comparing the potency of 8-epi-$PGF_{2\alpha}$ to that of its stereoisomere $PGF_{2\alpha}$ and a synthetic TxA_2 agonist, U46619. We administered 8-epi-$PGF_{2\alpha}$, $PGF_{2\alpha}$ and U46619 by nebulisation and assessed their bronchoconstrictive potencies by measurement of ventilatory mechanics.

2. MATERIALS AND METHODS

2.1. Horses

Six horses free from airway diseases (mean ± SD, 9.9 ± 4.9 years, 457 ± 21 kg bwt) and six horses suffering from heaves (mean ± SD, 16.7 ± 1.8 years, 491 ± 21 kg bwt) were used. The study was approved by the Animal Ethics Committee of the University of Liege.

The healthy individuals were chosen on the basis of their history, clinical examination and results of preliminary pulmonary function tests. Heaves-susceptible horses were selected on the basis of their response to allergen challenge by mouldy hay and the reversibility of airway obstruction by intravenous injection of atropine (0.04 $mg \cdot kg^{-1}$ bwt). The selected horses were investigated when they were in remission after a two-month period on pasture. They were admitted to the protocol if clinical examination and preliminary pulmonary function tests corresponded to a remission of heaves (Tab. I).

2.2. Preliminary pulmonary function tests

Pulmonary function tests included assessment of ventilatory mechanics, arterial blood gas tension and bronchoalveolar lavage (BAL) and were performed ten days preceding the protocol. At the time of the tests, all horses met the pulmonary function requirements and could be included in the study (Tab. I).

Table I. Preliminary pulmonary function tests. Values are presented as means ± SEM.

Variable (Unit)	*Healthy horses (n = 6)*	*Heaves-susceptible horses in remission (n = 6)*
BALF differential cell count		
Neutrophils (%)	3.5 ± 2.2	6.8 ± 4.7
Lymphocytes (%)	45.7 ± 12.3	42.5 ± 12.1
Macrophages (%)	50.1 ± 11.8	48.8 ± 5.6
Epithelial cells (%)	0.7 ± 1.6	1.9 ± 1.1
R_L ($kLa \cdot s \cdot L^{-1}$)	0.07 ± 0.01	0.09 ± 0.03
C_{dyn} ($L \cdot kPa^{-1}$)	15.3 ± 4.2	16.5 ± 3.3
Δpplmax (kPa)	0.67 ± 0.08	0.75 ± 0.12
PaO_2 (mmHg)	105 ± 3	97 ± 8

BALF: bronchoalveolar lavage fluid, R_L: total pulmonary resistance, C_{dyn}: dynamic lung compliance, Δpplmax: maximum variation of pleural pressure, PaO_2: arterial partial oxygen pressure.

Ventilatory mechanics required pleural pressure and respiratory airflow measurements. Intrapleural pressure was measured by means of an oesophageal balloon catheter made from a condom sealed over the end of a polyethylene catheter (4 mm inner diameter, 6 mm outer diameter, 220 cm, VEL, Leuven, Belgium) positioned with its tip in the middle thoracic oesophagus and connected to a pressure transducer (Valydine M1-45, Valydine Engineering, Northridge, CA, USA). A facemask covered the horse's nostrils and mouth. This mask was shaped in order to minimise dead space and to avoid nasal compression. A Fleisch pneumotachograph Nr. 4 mounted on the facemask was coupled with two catheters (4 mm inner diameter, 6 mm outer diameter, 220 cm, VEL, Leuven, Belgium) and a differential pressure transducer (Valydine DP45-18, Valydine Engineering, Northridge, CA, USA). Respiratory airflow and oesophageal pressure were simultaneously measured and total pulmonary resistance (R_L), dynamic compliance (C_{dyn}) and maximal pleural pressure changes (Δpplmax) were calculated on a breath-by-breath basis by a computer provided with lung function software (Po-Ne-Mah, Gould Instrument Systems, Valley View, OH, USA). Volume and pressure calibrations were performed with a 2 L pump (Medisoft, Dinant, Belgium) and a water manometer, respectively. More technical details are reported in [2]. The following limits were arbitrarily chosen to monitor wether the animals were healthy or in remission of heaves: $R_L \leq 0.11$ kPa·s·L^{-1}, $C_{dyn} \geq 10$ L·kPa^{-1} and Δpplmax ≤ 1.00 kPa.

Arterial blood was withdrawn anaerobically by puncture of *Arteria carotis communis* and analysed, after correction for body temperature, for partial pressure in O_2 (AVL 995, VEL, Leuven, Belgium). $PaO_2 \geq 90$ mm Hg was considered to be normal.

Bronchoalveolar lavage (BAL) was performed after the preliminary ventilatory mechanics measurement and arterial blood gas analysis on the sedated horse (Sedivet®, Boehringer Ingelheim, Ingelheim, Germany, romifidine, 0.01mg·kg^{-1} bwt iv) using a 250 cm fibreoptic endoscope (9 mm outer diameter) (Pentax, Breda, Netherlands) wedged in the bronchi, and by infusing at least 60 mL of saline previously heated at 37 °C. The dead space of the endoscope was already filled with saline allowing to infuse a small volume of saline. The fluid was recovered by gentle hand suction. The BAL was considered as successful when fluid was cloudy and alveolar surfactant could be recovered, indicating that the alveoli had been lavaged. Recovery of BAL fluid reached approximately 60%. A differential cell count of the BAL fluid was performed (Tab. I) and a neutrophil percentage lower than 10% in healthy horses and inferior to 12% in heaves-susceptible horses was considered acceptable.

2.3. Prostaglandin challenges and ventilatory mechanics measurements

2.3.1. Experimental design

Prostaglandin challenges started ten days after the preliminary tests in order to ensure that the potential airway irritations due to endoscopy completely resolved. Each horse underwent four nebulisation challenges, in a randomised order for four consecutive days. Nebulisations consisted in a single dose either of placebo (NaCl 0.9 % + 100 µL ethanol) or a dose of 1 mg of 8-epi-$PGF_{2\alpha}$, $PGF_{2\alpha}$ and synthetic thromboxane agonist U46619 (respectively 2.83 µM, 2.83 µM and 2.85 µM). Measurement of pulmonary mechanics were performed immediately before (T_{Ante}) and for five minutes after (T_{Post}) the inhalation challenge.

2.3.2. Drugs

8-Epi-$PGF_{2\alpha}$, $PGF_{2\alpha}$ and thromboxane A_2 agonist U46619 were purchased from

Cayman Chemical (Abingdon, UK). Drugs were dissolved in ethanol (99%) and divided into 100 µL aliquots containing 1 mg of agonist. Aliquots were stored at –20 °C until use and were prepared with 4 mL saline (0.9%). Preliminary experiments showed that the addition of 100 µL ethanol to 4 mL saline did not influence the mechanics of breathing. The choice of the single dose of 1 mg 8-epi-$PGF_{2\alpha}$, $PGF_{2\alpha}$ or U46619 was made on the basis of the following reasoning: (1) The excessive costs of the compounds used in this study made it impossible to perform the classical dose-response reactivity tests [1, 5, 8, 29] and it was decided to use comparative single dose challenges. (2) By comparing the effect of identical doses of 8-epi-$PGF_{2\alpha}$ to that of $PGF_{2\alpha}$ and U46619, the establishment of a rank order of potency was possible. As all compounds tested were increased in vivo to a similar extent by an acute crisis of heaves, the rank order established by our study should correspond to their physiological potency. (3) Preliminary tests had shown that $PGF_{2\alpha}$ was the most potent bronchoconstrictor, and the highest dose which was tolerated by the heaves-susceptible horses was selected as a reference dose for all compounds.

2.3.3. Experimental procedure

Prior to nebulisation, baseline values of ventilatory mechanics (T_{Ante}) were recorded for two minutes by the same method as described before. The facemask was removed for nebulisation, whereas the oesophageal balloon catheter (introduced through the right nostril) remained in place. An ultrasonic nebuliser (DeVilbiss Ultraneb® 2000, Springfield, OH, USA) suitable for the horse's lower airway nebulisation was used for aerosol generation [30]. Aerosolised placebo or drugs were administered for two minutes (i.e. the time necessary for aerosolisation of 4 mL) through a plastic tube (length 60 cm, diameter 3 cm) and a nostril-piece tightly shaped into the left nostril. The aerosol delivery occurred by constant positive flow through the tube and the nostril-piece, and the horse inspired aerosol through the left nostril and fresh air through the right nostril. Immediately after administration of drugs, the nebulisation system was removed and the facemask replaced on the horse's head. Ventilatory mechanic measurements started one minute after the end of the challenge and were performed for five consecutive minutes after the challenge (T_{Post}). Preliminary tests had shown that the onset of bronchoconstriction occurred during or immediately after the nebulisation of drugs. Maximum bronchoconstriction was observed within one to two minutes after the beginning of the ventilatory mechanics recording and a plateau was maintained during at least four and a maximum of seven minutes. The mean R_L and C_{dyn} values of a five minute recording most accurately reflected the drug-induced modifications. Post-inhalation measurements reached baseline values within twenty minutes after the challenge. Delayed bronchoconstriction or carry-over effects were not observed.

2.4. Data analysis

Ventilatory mechanics data (R_L, C_{dyn}) are presented as means ± standard error of mean (SEM). Measurements taken before (baseline value, T_{Ante}) and after (T_{Post}) inhalation challenge were averaged per horse and per group (healthy and heaves-susceptible horses). An analysis of variance (ANOVA) for repeated measures was used for comparison between T_{Ante} and T_{Post} values of inhaled drugs within each group. The comparison of responses between the placebo and each agonist within groups and the comparison of responses between healthy and heaves-susceptible horses were performed by one-way ANOVA. The limit of significance was set at $P < 0.05$.

As the heaves-susceptible horses were significantly older than the healthy horses

(age range of heaves-susceptible horses: 15-20 years, age range of healthy horses: 3-14 years, $P = 0.04$, unpaired T-test), the potential effect of age on both respiratory variables (C_{dyn} and R_L) was analysed by linear regression. The T_{Ante} and T_{Post} values of C_{dyn} and R_L recorded at each nebulisation challenge of healthy and heaves-susceptible horses were pooled and correlated with the horses' age.

3. RESULTS

Inhalation of placebo did not affect ventilatory mechanics (R_L and C_{dyn}), neither in healthy horses (Fig. 1A and Fig. 2A) nor in heaves-susceptible horses (Fig. 1B and Fig. 2B). Furthermore, there were no significant differences between baseline values assessed before each nebulisation challenge, neither between healthy and heaves-susceptible horses nor between different challenges within groups.

Inhalation of 8-epi-$PGF_{2\alpha}$ did not significantly change baseline values of R_L and C_{dyn} in healthy horses (Fig. 1A and Fig. 2A). In heaves-susceptible horses, both R_L and C_{dyn} were significantly changed by 8-epi-$PGF_{2\alpha}$ inhalation, but R_L increase and C_{dyn} decrease were not significantly different from respective placebo values (Fig. 1B and Fig. 2B). The R_L and C_{dyn} post-inhalation values of healthy and heaves-susceptible horses were not significantly different.

Administration of $PGF_{2\alpha}$ induced a significant increase of R_L and a significant decrease of C_{dyn} in healthy horses, but R_L and C_{dyn} values were not significantly different from those of placebo inhalation (Fig. 1A and Fig. 2A). Heaves-susceptible horses showed a significant increase of R_L and a significant decrease of C_{dyn} when compared to baseline values, placebo and 8-epi-$PGF_{2\alpha}$ responses (Fig. 1B and Fig. 2B). Furthermore, R_L increase of the heaves-susceptible group was significantly higher than that of the healthy group.

The thromboxane agonist U46619 induced a significant increase of R_L in healthy horses, whereas C_{dyn} was not significantly decreased (Fig. 1A and Fig. 2A). The increase of R_L was not significantly greater than that after placebo inhalation. In heaves-susceptible horses, both R_L and C_{dyn} values were significantly different from those of the baseline, and only the R_L increase was significantly greater than that after placebo inhalation (Fig. 1B and Fig. 2B). R_L and C_{dyn} values of healthy horses were not significantly different from those of heaves-susceptible horses.

The regression analyses between age and R_L or C_{dyn} values revealed no significant correlation.

4. DISCUSSION

Our study aimed at evaluating the bronchoconstrictive properties of inhaled 8-epi-$PGF_{2\alpha}$ in healthy and heaves-susceptible horses by comparing effects of a single dose of 8-epi-$PGF_{2\alpha}$ with those of $PGF_{2\alpha}$ and U46619. By contrast to previous in vivo and in vitro studies performed in human and rodent lung tissues [3, 17, 25], 8-epi-$PGF_{2\alpha}$ was not a bronchoconstrictor in healthy horses at the dose tested (1 mg). Airway responsiveness to 8-epi-$PGF_{2\alpha}$ of heaves-susceptible horses was higher, but not significantly different from that of healthy horses. Heaves-susceptible horses were hyperresponsive to $PGF_{2\alpha}$ when compared with healthy horses. In heaves-susceptible horses, the inhalation of U46619 induced bronchospasm, which was significantly different from that of placebo inhalation.

We compared the bronchoconstrictive effects of a single dose of 8-epi-$PGF_{2\alpha}$ to those of identical doses of $PGF_{2\alpha}$ and U46619, a synthetic TxA_2 analog. The limiting factor of this experimental design is the use of a single dose rather than incremental doses allowing the construction of a classical dose-response curve [1, 5, 8, 29]. Our results allow to establish a rank order of

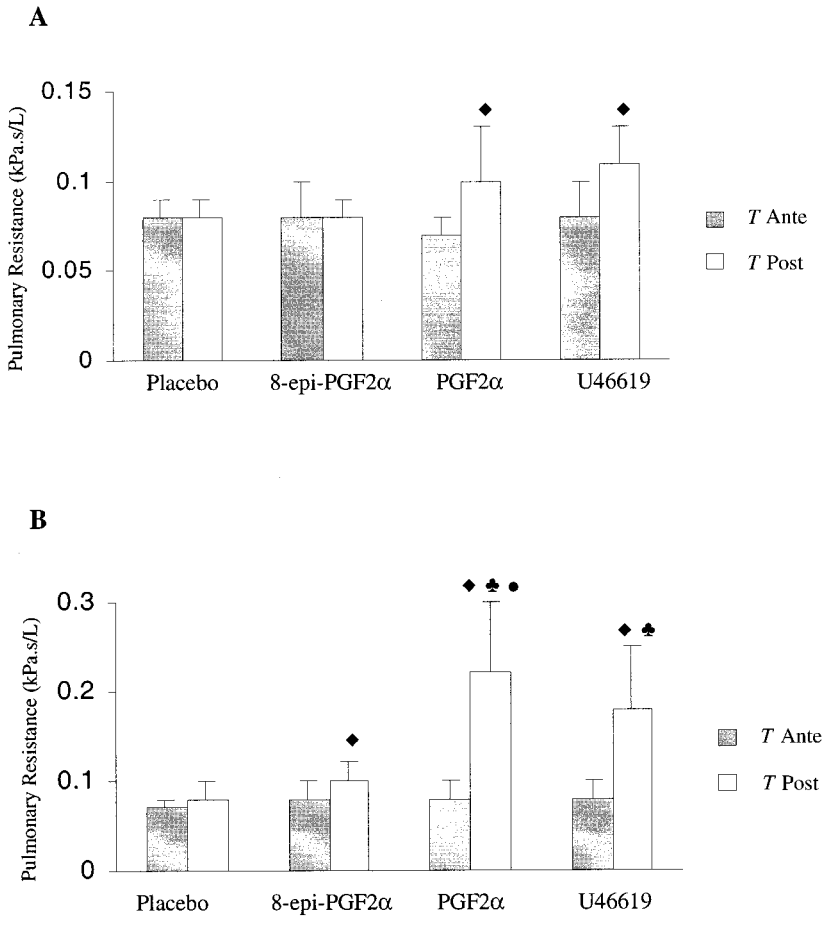

Figure 1. Response of pulmonary resistance (R_L) to inhalation challenge (placebo, 8-epi-$PGF_{2\alpha}$, $PGF_{2\alpha}$, U46619) in healthy horses, $n = 6$ (**A**) and in heaves-susceptible horses, $n = 6$ (**B**).
◆ significantly different from the respective T_{Ante} value, ♣ significantly different from placebo T_{Post} value, ● significantly different from the 8-epi-$PGF_{2\alpha}$ T_{Post} value.
Data are presented as mean ± SEM.

potency of the three agonists tested at the dose of 1 mg (8-epi-$PGF_{2\alpha}$ < U46619 < $PGF_{2\alpha}$), but whether higher doses of 8-epi-$PGF_{2\alpha}$ would change this rank order is unknown. Preliminary tests with lower doses (0.5 mg) of each agonist have shown that no changes of rank order occurred, neither in healthy, nor in heaves-susceptible horses (unpublished data). Dose-response curves established on lung tissues of other species indicate that the 8-epi-$PGF_{2\alpha}$ curve is a sigmoidal curve [3, 17, 25]. It is therefore probable that the same would be true in horses, but we were unable to prove this assumption by our study.

The decision to administer 1 mg of agonist was taken on the basis of the preliminary results, showing that

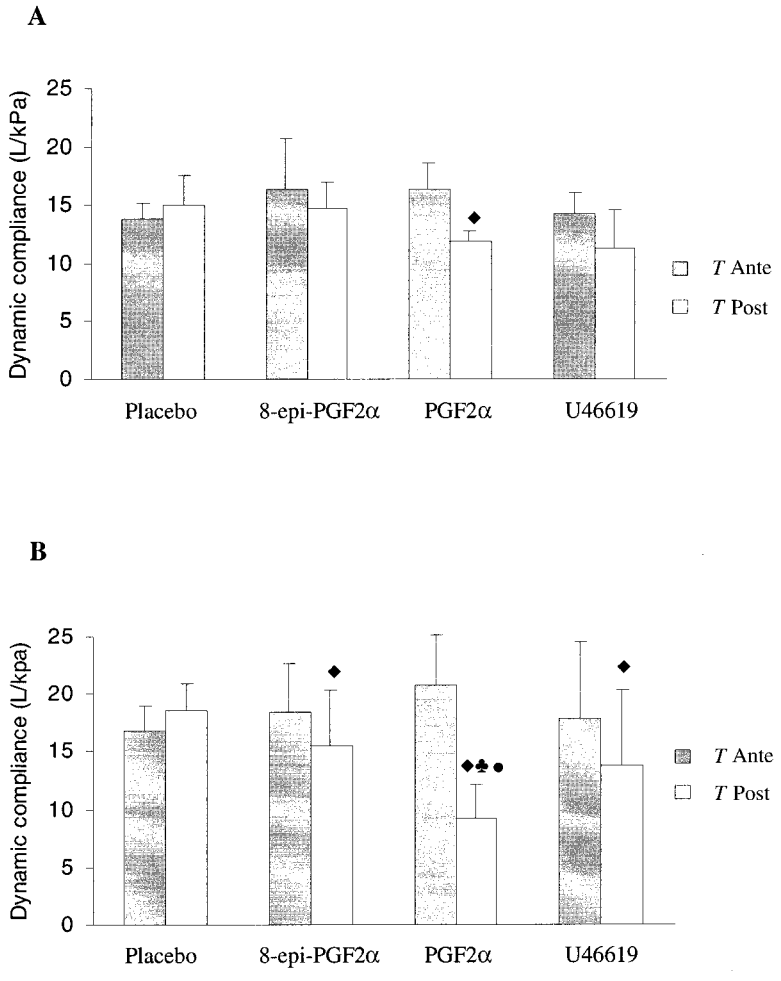

Figure 2. Response of dynamic compliance (C_{dyn}) to inhalation challenge (placebo, 8-epi-$PGF_{2\alpha}$, $PGF_{2\alpha}$, U46619) in healthy horses, $n = 6$ (**A**) and in heaves-susceptible horses, $n = 6$ (**B**).
◆ significantly different from the respective T_{Ante} value, ♣ significantly different from placebo T_{Post} value, ● significantly different from 8-epi-$PGF_{2\alpha}$ T_{Post} value.
Data are presented as mean ± SEM.

heaves-susceptible horses in remission suffer from severe bronchospasm after being nebulised by 1 mg of $PGF_{2\alpha}$ and do not tolerate nebulisation of greater amounts of $PGF_{2\alpha}$. In heaves-susceptible horses, $PGF_{2\alpha}$ and the synthetic TxA_2 analog, U46619, induced significant bronchoconstriction, which was in agreement with previous in vitro studies performed on equine trachealis muscle and lung parenchyma [9] and which indicates that $PGF_{2\alpha}$ and TxA_2 might play an important role in airway obstruction occurring during an acute crisis of heaves. Both prostanoids are increased in BAL of heaves-susceptible horses in acute crisis [12, 31], but despite non-steroidal anti-

inflammatory treatment, airway obstruction has been shown to persist, suggesting a minor role for arachidonic metabolites in heaves [12]. This implies that 8-epi-$PGF_{2\alpha}$, which induced slight but significant bronchospasm and which undergoes a relative increase similar to that of $PGF_{2\alpha}$ and TxA_2 in BAL of heaves-susceptible horses in crisis, is a much less powerful bronchoconstrictor than $PGF_{2\alpha}$ and TxA_2. This is in agreement with earlier in vitro studies which demonstrated, by receptor antagonism, that 8-epi-$PGF_{2\alpha}$ mainly acts as a partial agonist through the thromboxane TP receptor and not through the $PGF_{2\alpha}$ FP receptor [3, 11, 17, 25]. Even if non-steroidal anti-inflammatory treatment does not inhibit 8-epi-$PGF_{2\alpha}$ synthesis [27], the potency of 8-epi-$PGF_{2\alpha}$ is low and 8-epi-$PGF_{2\alpha}$ appears to be an oxidative damage marker rather than a significant bronchoconstrictor.

Heaves-susceptible horses showed a stronger airway response to all inhaled agonists, inhalation of $PGF_{2\alpha}$ induced even a significantly higher R_L than in healthy horses. These results suggest that heaves-susceptible horses in clinical remission are hyperresponsive to inhaled $PGF_{2\alpha}$, which is in disagreement with previous studies using histamine, methacholine and citric acid as an airway challenge [1, 5, 8, 29]. The authors of these studies have shown that heaves-susceptible horses were suffering from non-specific AHR only when they were in crisis or housed in a controlled environment, but not when they were in remission after a two-month period on pasture. In heaves-susceptible horses, AHR is believed to be related to pulmonary inflammation [28]. As shown in Table I, no significant difference was observed in the BAL differential cell count, especially in neutrophil percentage, between healthy and heaves-susceptible horses. The AHR of the heaves-susceptible horses to inhaled $PGF_{2\alpha}$ was apparently not due to neutrophilic airway inflammation and it might be hypothesised that it was a specific response of heaves-susceptible horses to prostaglandins, especially to $PGF_{2\alpha}$. In asthmatic humans, factors other than inflammation have been shown to be critical for AHR, e.g. airway remodelling, epithelial injury, airway smooth muscle contractility… [4, 6, 26]. Pulmonary neutrophils and lymphocytes (Th1 and Th2) play key roles in asthma [13, 15]. In heaves, neutrophils have been demonstrated to be a cornerstone in the inflammatory cascade and airway obstruction [7], but little is known about the role of lymphocytes in the development of the disease [28]. Airway remodelling encompasses all morphological modifications encountered in asthma and is a consequence of chronic airway inflammation [10]. The irreversible structural modifications are thought to play an important role in generating the symptoms of asthma, including AHR. In heaves-susceptible horses, morphological modifications of the airways, similar to those observed in human asthma, are reported [16, 28]. The impact of these changes on AHR has never been investigated in horses and the possibility of AHR as a consequence of airway remodelling might be considered. As the heaves-susceptible horses used in our study were significantly older than the healthy horses, the impact of the horse's age on AHR was assessed by regression analysis. The results did not confirm a link between age and increased AHR, and the possibility of age-dependent airway remodelling causing AHR could be rejected.

Taken together, our results allow to conclude that 8-epi-$PGF_{2\alpha}$ seems to play a minor role in airway obstruction occurring in heaves. 8-Epi-$PGF_{2\alpha}$ should be considered as an oxidative damage marker rather than an oxidative bronchoconstrictive actor.

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